

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of all claims in the application.

Listing of Claims

1. **(Original)** A method of screening a composition for non-receptor-specific G-protein coupled receptor (GPCR) desensitization inhibitory activity comprising the steps of:
 - (a) providing a first cell comprising a first GPCR and a first conjugate of a marker molecule and a protein associated with the desensitization pathway of the first GPCR, the first GPCR being a GPCR that requires an agonist for desensitization or being a constitutively desensitized GPCR;
 - (b) exposing the first cell to a test composition and, when the first GPCR requires agonist for desensitization, to an agonist for the first GPCR;
 - (c) determining, through detection of the marker molecule in the first conjugate, whether or not the composition gives an indication of GPCR desensitization inhibitory activity with respect to the first GPCR;
 - (d) providing a second cell comprising a second GPCR different from the first GPCR and a second conjugate of a marker molecule and a protein associated with the desensitization pathway of the second GPCR, the second conjugate being the same or different from the first conjugate, the second GPCR being a GPCR that requires an agonist for desensitization or being a constitutively desensitized GPCR;
 - (e) exposing the second cell to the test composition and, when the second GPCR requires agonist for desensitization, to an agonist for the second GPCR;
 - (f) determining, through detection of the marker molecule in the second conjugate, whether or not the composition gives an indication of GPCR desensitization inhibitory activity with respect to the second GPCR; wherein an indication of GPCR desensitization inhibitory activity for the test composition in both step (c) and step (f) being an indication

that the test composition has non-receptor-specific GPCR desensitization inhibitory activity.

2. **(Original)** The method of claim 1, wherein the protein in the first conjugate associated with the desensitization pathway of the first GPCR is selected from the group consisting of an arrestin protein and the first GPCR and wherein the protein in the second conjugate associated with the desensitization pathway of the second GPCR is selected from the group consisting of an arrestin protein and the second GPCR.
3. **(Original)** The method of claim 1, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.
4. **(Original)** The method of claim 2, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.
5. **(Original)** The method of claim 1, wherein: step (c) comprises detecting for translocation or localization of the first conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity; and step (f) comprises detecting for translocation or localization of the second conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity.
6. **(Original)** The method of claim 1, wherein: step (c) comprises detecting for translocation or localization of the first conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity; and step (f) comprises detecting for translocation or localization of the second conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity.

7. **(Original)** The method of claim 1, wherein: step (c) comprises detecting for translocation or localization of the first conjugate, a decreased level of translocation or localization with respect to a level of translocation or localization determined in a control cell not exposed to the test composition being an indication that the composition has GPCR desensitization inhibitory activity, the control cell comprising the first GPCR and the first conjugate and being exposed to agonist if the first GPCR requires agonist for desensitization; and step (f) comprises detecting for translocation or localization of the second conjugate, a decreased level of translocation or localization with respect to a level of translocation or localization determined in a control cell not exposed to the test composition being an indication that the composition has GPCR desensitization inhibitory activity, the control cell comprising the second GPCR and the second conjugate and being exposed to agonist if the second GPCR requires agonist for desensitization.

8. **(Original)** The method of claim 1, wherein: the first GPCR is a GPCR that requires agonist for desensitization; in step (b), the first cell is exposed to the test composition first and then is exposed to the agonist for the first GPCR; the second GPCR is a GPCR that requires agonist for desensitization; and in step (e), the second cell is exposed to the test composition first and then is exposed to the agonist for the second GPCR.

9. **(Original)** The method of claim 1, wherein: the first GPCR is a GPCR that requires agonist for desensitization; in step (b), the first cell is exposed to the agonist for the first GPCR and then is exposed to the test composition; the second GPCR is a GPCR that requires agonist for desensitization; and in step (e), the second cell is exposed to the agonist for the second GPCR and then is exposed to the test composition.

10. **(Original)** The method of claim 1, wherein: in the first conjugate, the protein associated with the desensitization pathway of the first GPCR comprises an arrestin protein and the marker molecule comprises a GFP; in the second conjugate, the protein associated with the desensitization pathway of the second GPCR comprises an arrestin protein and the marker molecule comprises a GFP; the first GPCR is a GPCR that

requires agonist for desensitization; the second GPCR is a GPCR that requires agonist for desensitization; in step (b), the first cell is exposed to the test composition first and then is exposed to the agonist for the first GPCR; step (c) comprises detecting for translocation or localization of the first conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity; in step (e), the second cell is exposed to the test composition first and then is exposed to the agonist for the second GPCR; and step (f) comprises detecting for translocation or localization of the second conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity.

11. **(Original)** The method of claim 1, wherein: the first conjugate comprises the first GPCR conjugated to the marker molecule; the second conjugate comprises the second GPCR conjugated to the marker molecule; step (c) comprises detecting for translocation or localization of the first GPCR, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity; and step (f) comprises detecting for translocation or localization of the second GPCR, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity.

12. **(Original)** The method of claim 1, wherein: the first conjugate comprises the first GPCR conjugated to the marker molecule; the second conjugate comprises the second GPCR conjugated to the marker molecule; step (c) comprises detecting for translocation or localization of the first GPCR, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity; and step (f) comprises detecting for translocation or localization of the second GPCR, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity.

13. **(Original)** A method of screening a composition for non-receptor-specific G-protein coupled receptor (GPCR) desensitization inhibitory activity comprising the steps of:

- (a) providing a first cell comprising a first GPCR that requires agonist for desensitization and a first conjugate of a marker molecule and a protein associated with the desensitization pathway of the first GPCR;
- (b) exposing the first cell to a test composition and to an agonist for the first GPCR;
- (c) determining whether or not the composition gives an indication of GPCR desensitization inhibitory activity with respect to the first GPCR by detecting for translocation or localization of the first conjugate, the following being an indication that the composition has GPCR desensitization inhibitory activity:
 - (1) a lack of translocation or localization;
 - (2) a decrease in translocation or localization after exposure to the test composition; or
 - (3) a decreased level of translocation or localization with respect to a predetermined level or with respect to a level determined in a control cell not exposed to the test composition, the control cell comprising the first GPCR and the first conjugate and being exposed to the agonist for the first GPCR;
- (d) providing a second cell comprising a second GPCR that requires agonist for desensitization and is different from the first GPCR and a second conjugate of a marker molecule and a protein associated with the desensitization pathway of the second GPCR, the second conjugate being the same or different from the first conjugate;
- (e) exposing the second cell to the test composition and to an agonist for the second GPCR;
- (f) determining whether or not the composition gives an indication of GPCR desensitization inhibitory activity with respect to the second GPCR by detecting for translocation or localization of the second conjugate, the following being an indication that the composition has GPCR desensitization inhibitory activity:
 - (1) a lack of translocation or localization;

(2) a decrease in translocation or localization after exposure to the test composition; or

(3) a decreased level of translocation or localization with respect to a predetermined level or with respect to a level determined in a control cell not exposed to the test composition, the control cell comprising the second GPCR and the second conjugate and being exposed to the agonist for the second GPCR;

wherein an indication of GPCR desensitization inhibitory activity for the test composition in both step (c) and step (f) being an indication that the test composition has non-receptor-specific GPCR desensitization inhibitory activity.

14. **(Original)** The method of claim 13, wherein the protein in the first conjugate associated with the desensitization pathway of the first GPCR is selected from the group consisting of an arrestin protein and the first GPCR and wherein the protein in the second conjugate associated with the desensitization pathway of the second GPCR is selected from the group consisting of an arrestin protein and the second GPCR.

15. **(Original)** The method of claim 14, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

16. **(Original)** The method of claim 15, wherein: in the first conjugate, the protein associated with the desensitization pathway of the first GPCR comprises an arrestin protein and the marker molecule comprises a GFP; and in the second conjugate, the protein associated with the desensitization pathway of the second GPCR comprises an arrestin protein and the marker molecule comprises a GFP.

17. **(Original)** The method of claim 16, wherein, when there is an indication that the test composition has non-receptor-specific GPCR desensitization inhibitory activity,

the method further comprises performing a kinase assay to determine whether the test composition is a kinase inhibitor.

18. **(Original)** The method of claim 17, wherein, when there is an indication that the test composition has non-receptor-specific GPCR desensitization inhibitory activity, the method further comprises performing a GPCR ligand binding assay to confirm that the GPCR desensitization inhibitory activity of the test composition is not receptor specific.

19. **(Original)** A method of screening a composition for non-receptor-specific G-protein coupled receptor (GPCR) desensitization inhibitory activity comprising the steps of:

(a) providing a cell comprising:

- (1) a first GPCR that is a GPCR that requires agonist for desensitization;
- (2) a second GPCR that is different than the first GPCR, the second GPCR being a GPCR that requires agonist for desensitization;
- (3) a first conjugate of a marker molecule and a protein associated with the desensitization pathway of the first GPCR; and
- (4) a second conjugate of a marker molecule and a protein associated with the desensitization pathway of the second GPCR, the second conjugate being the same or different from the first conjugate;

(b) exposing the cell to a test composition and to an agonist for the first GPCR;

(c) determining whether or not the composition has GPCR desensitization inhibitory activity with respect to the first GPCR;

(d) exposing the cell to an agonist for the second GPCR and optionally re-exposing the cell to the test composition; and

(e) determining whether or not the composition has GPCR desensitization inhibitory activity with respect to the second GPCR; wherein an indication that the test composition has GPCR desensitization inhibitory activity with respect to both the first GPCR and the

second GPCR being an indication that the test composition has non-receptor-specific GPCR desensitization inhibitory activity; and wherein the agonist for the first GPCR is not an agonist for the second GPCR and the agonist for the second GPCR is not an agonist for the first GPCR.

20. **(Original)** The method of claim 19, wherein the protein in the first conjugate associated with the desensitization pathway of the first GPCR is selected from the group consisting of an arrestin protein and the first GPCR and wherein the protein in the second conjugate associated with the desensitization pathway of the second GPCR is selected from the group consisting of an arrestin protein and the second GPCR.

21. **(Original)** The method of claim 19, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

22. **(Original)** The method of claim 20, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

23. **(Original)** The method of claim 19, wherein: step (c) comprises detecting for translocation or localization of the conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR; and step (e) comprises detecting for translocation or localization of the conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR.

24. **(Original)** The method of claim 19, wherein: step (c) comprises detecting for translocation or localization of the conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR; and step (e) comprises

detecting for translocation or localization of the second conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR.

25. **(Original)** The method of claim 19, wherein: step (c) comprises detecting for translocation or localization of the conjugate, a decreased level of translocation or localization with respect to a level of translocation or localization determined in a control cell not exposed to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR, the control cell comprising the first GPCR and the conjugate and being exposed to agonist if the first GPCR requires agonist for desensitization; and step (e) comprises detecting for translocation or localization of the conjugate, a decreased level of translocation or localization with respect to a level of translocation or localization determined in a control cell not exposed to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR, the control cell comprising the second GPCR and the conjugate and being exposed to agonist if the second GPCR requires agonist for desensitization.

26. **(Original)** A method of screening a composition for non-receptor-specific G-protein coupled receptor (GPCR) desensitization inhibitory activity comprising the steps of:

(a) providing a cell comprising:

(1) a first GPCR that is a GPCR that requires agonist for desensitization or is a constitutively desensitized GPCR;

(2) a second GPCR that is different than the first GPCR, the second GPCR being a GPCR that requires agonist for desensitization or being a constitutively desensitized GPCR;

(3) a first conjugate of a first marker molecule and a protein associated with the desensitization pathway of the first GPCR; and

(4) a second conjugate of a second marker molecule and a protein associated with the desensitization pathway of the second GPCR, the second conjugate being different from the first conjugate; wherein the protein of the first conjugate is not included in the desensitization pathway of the second GPCR, the protein in the second conjugate is not included in the desensitization pathway of the first GPCR, and the first and second marker molecules are different from each other and are distinguishable from each other upon detection;

(b) exposing the cell

(1) to a test composition,

(2) when the first GPCR requires agonist for desensitization, to an agonist for the first GPCR, and

(3) when the second GPCR requires agonist for desensitization, to an agonist for the second GPCR; and

(c) determining whether or not the composition has GPCR desensitization inhibitory activity with respect to the first GPCR and with respect to the second GPCR, wherein an indication that the test composition has GPCR desensitization inhibitory activity with respect to both the first GPCR and the second GPCR being an indication that the test composition has non-receptor-specific GPCR desensitization inhibitory activity.

27. **(Original)** The method of claim 26, wherein: the first conjugate comprises the first GPCR conjugated to the first marker molecule; and the second conjugate comprises the second GPCR conjugated to the second marker molecule.

28. The method of claim 26, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

29. **(Original)** The method of claim 27, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected

from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

30. **(Original)** The method of claim 26, wherein step (c) comprises: (1) detecting for translocation or localization of the first conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR; and (2) detecting for translocation or localization of the second conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR.

31. **(Original)** The method of claim 26, wherein step (c) comprises: (1) detecting for translocation or localization of the first conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR; and (2) detecting for translocation or localization of the second conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR.

32. **(Original)** The method of claim 26, wherein step (c) comprises: (1) detecting for translocation or localization of the first conjugate, a decreased level of translocation or localization with respect to a level of translocation or localization determined in a control cell not exposed to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR, the control cell comprising the first GPCR and the first conjugate and being exposed to agonist if the first GPCR requires agonist for desensitization; and (2) detecting for translocation or localization of the second conjugate, a decreased level of translocation or localization with respect to a level of translocation or localization determined in a control cell not exposed to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR, the control cell

comprising the second GPCR and the second conjugate and being exposed to agonist if the second GPCR requires agonist for desensitization.

33. **(Original)** The method of claim 26, wherein: the first GPCR is a GPCR that requires agonist for desensitization; the second GPCR is a GPCR that requires agonist for desensitization; and in step (b), the cell is exposed to the test composition before the cell is exposed to the agonist for the first GPCR or to the agonist for the second GPCR.

34. **(Original)** The method of claim 26, wherein: the first conjugate comprises the first GPCR conjugated to the first marker molecule; the second conjugate comprises the second GPCR conjugated to the second marker molecule; and step (c) comprises: (1) detecting for translocation or localization of the first conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR; and (2) detecting for translocation or localization of the second conjugate, a lack of translocation or localization being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR.

35. **(Original)** The method of claim 26, wherein: the first conjugate comprises the first GPCR conjugated to the first marker molecule; the second conjugate comprises the second GPCR conjugated to the second marker molecule; and step (c) comprises: (1) detecting for translocation or localization of the first conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the first GPCR; and (2) detecting for translocation or localization of the second conjugate, a decrease in translocation or localization after exposure to the test composition being an indication that the composition has GPCR desensitization inhibitory activity with respect to the second GPCR.